

Supplementary material to the paper:

A new lineage of non-photosynthetic green algae with extreme organellar genomes

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This file contains supplementary Figs S1 to S8.

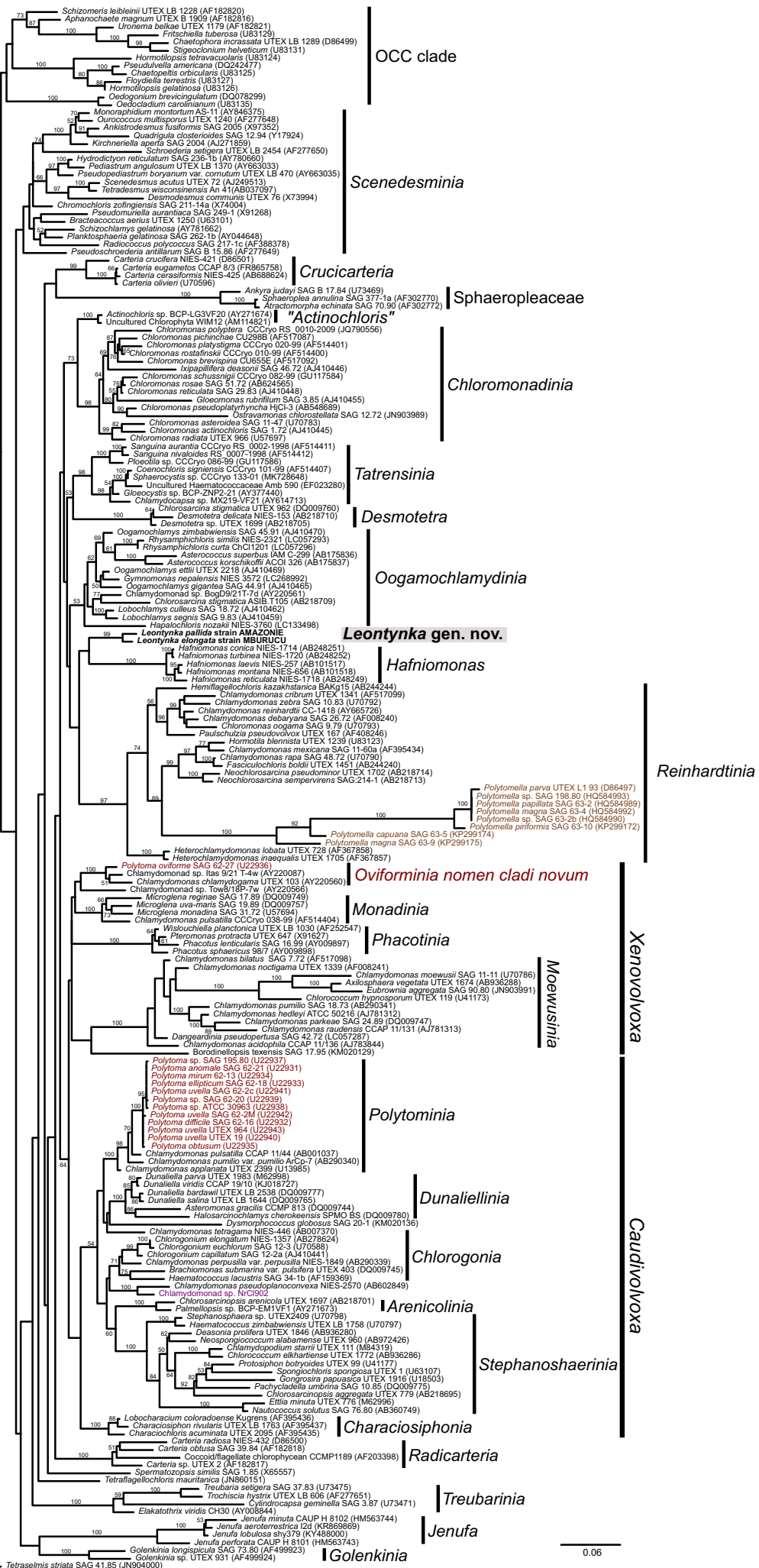


Fig. S1 Maximum likelihood phylogenetic tree (IQ-TREE, TIM2+F+I+G4 substitution model) of 18S rRNA gene sequences from Chlorophyceae. The chlorodendrophycean *Tetraselmis striata* is used as an outgroup. Bootstrap support values are shown when ≥ 50 . Previously demarcated main clades (Nakada et al., 2008) are annotated in the tree together with the newly designated clade "*Oviforminia*". Sequences from non-photosynthetic taxa are in colour. The new genus *Leontynka* (highlighted) forms a novel clade without apparent specific affinities to other particular lineages of Chlamydomonadales. The clade "*Actinochloris*" is labelled provisionally, as the bona fide *Actinochloris* genus belongs elsewhere.

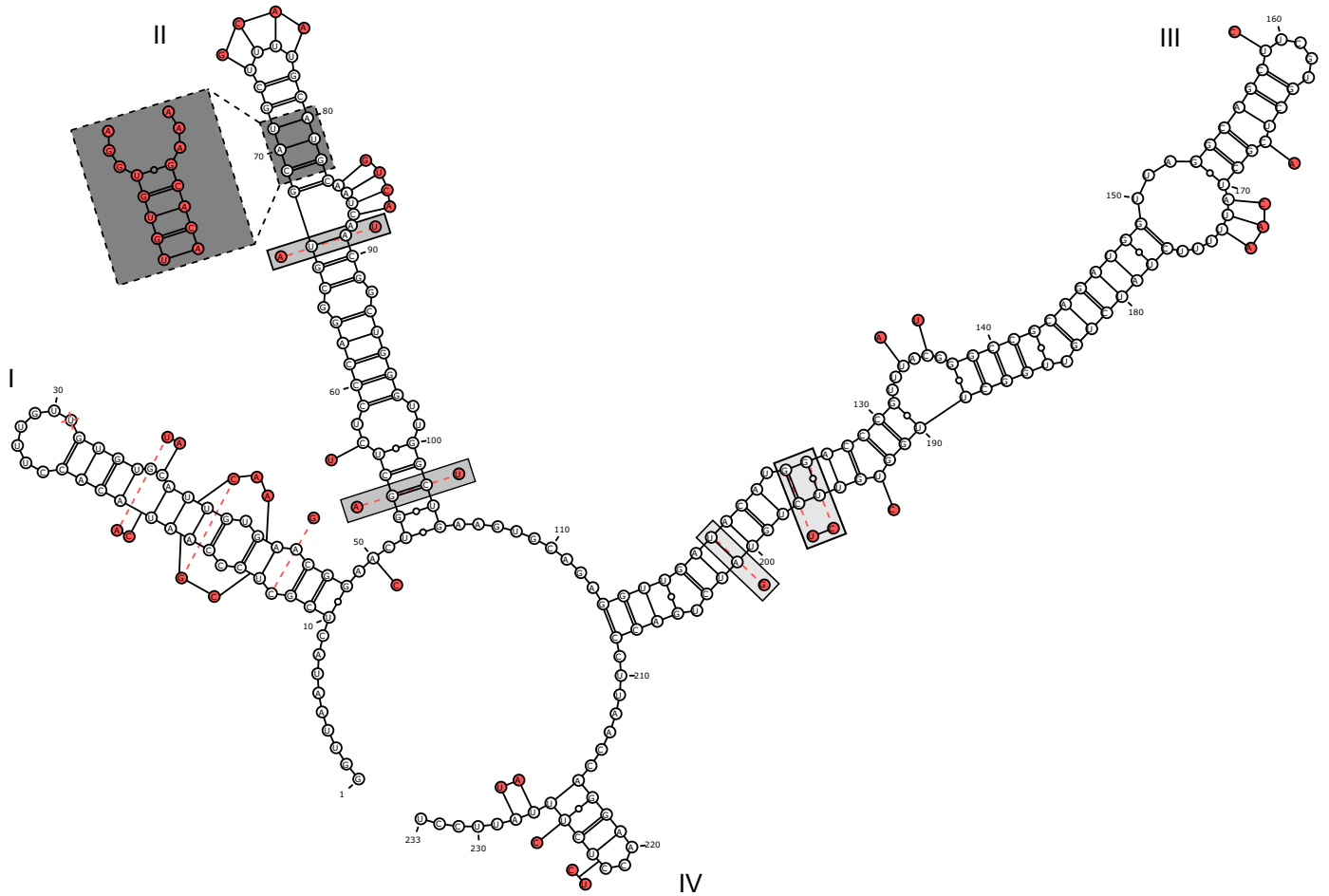


Fig. S2 Predicted secondary structure of the ITS2 region of *Leontynka pallida*, with differences in the corresponding region of *Leontynka elongata* mapped onto it. Classical compensatory base changes in the helix II are highlighted by a light grey background, a region more substantially differing between the two species is highlighted by a dark grey background.

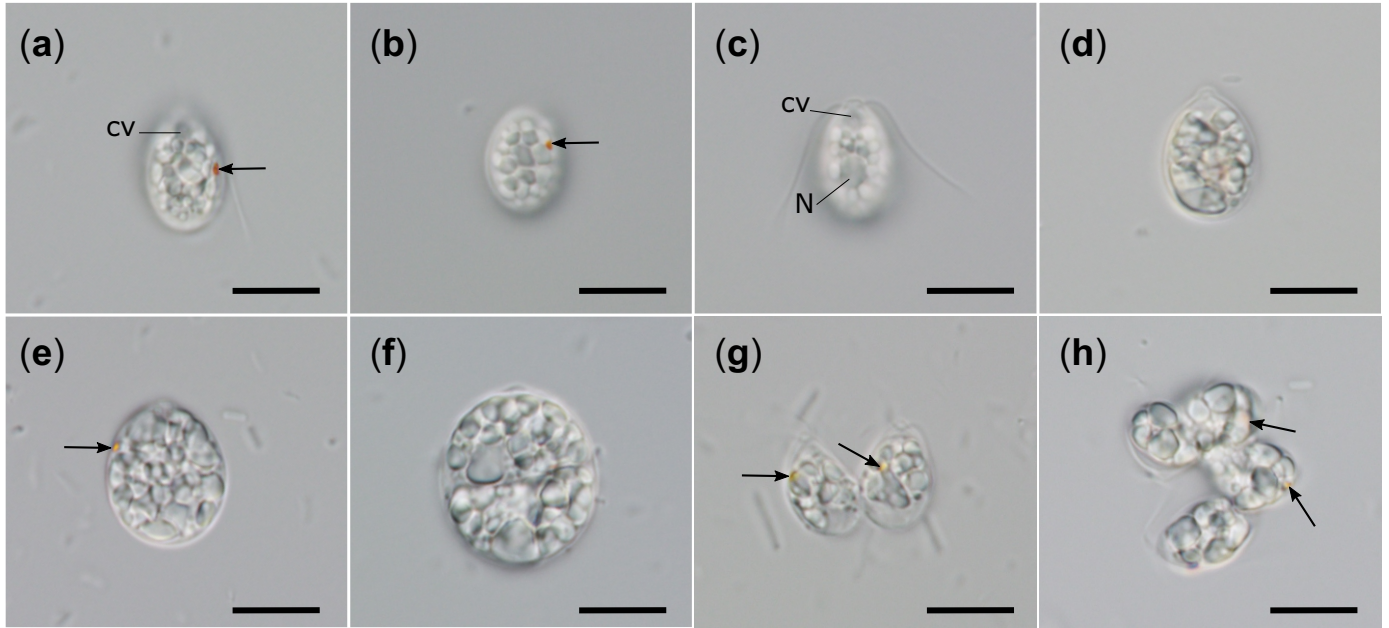


Fig. S3 *Leontynka pallida* under the light microscope. Scale bars = 10 µm.
 Abbreviations: arrows – eyespot; cv – contractile vacuole; N – nucleus.

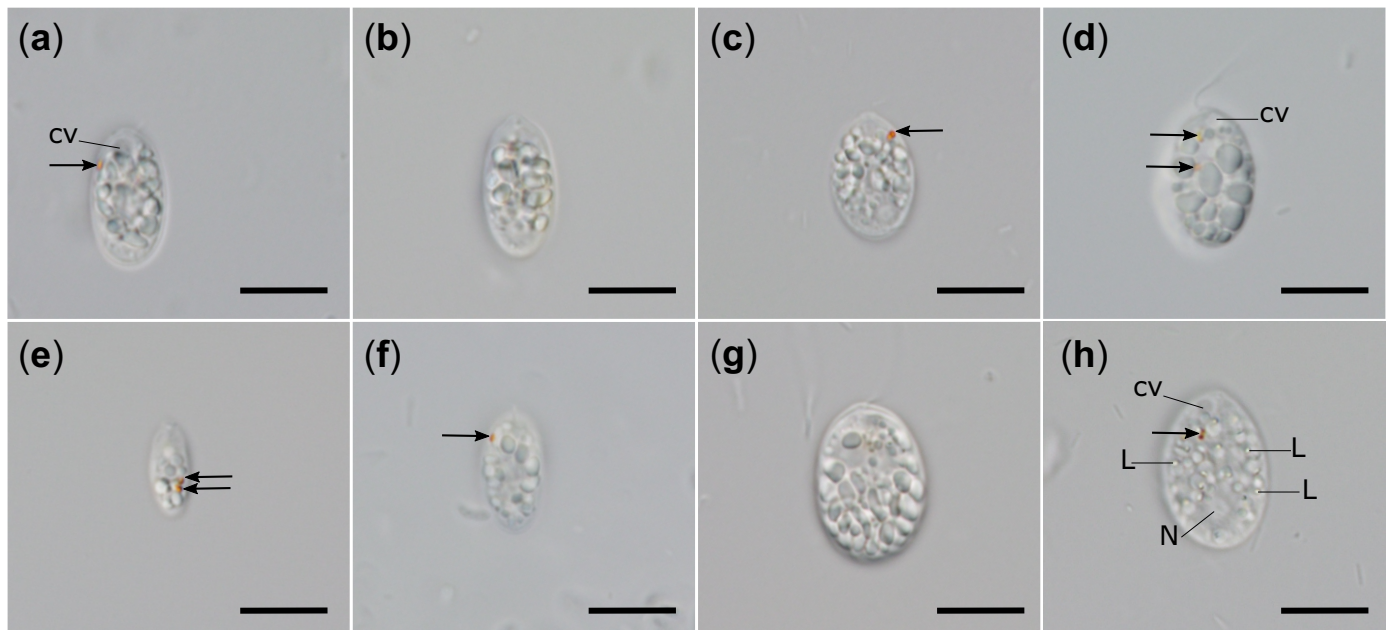


Fig. S4 *Leontynka elongata* under the light microscope. Scale bars = 10 µm.
 Abbreviations: arrows – eyespot; cv – contractile vacuole; L – lipid droplet; N – nucleus.

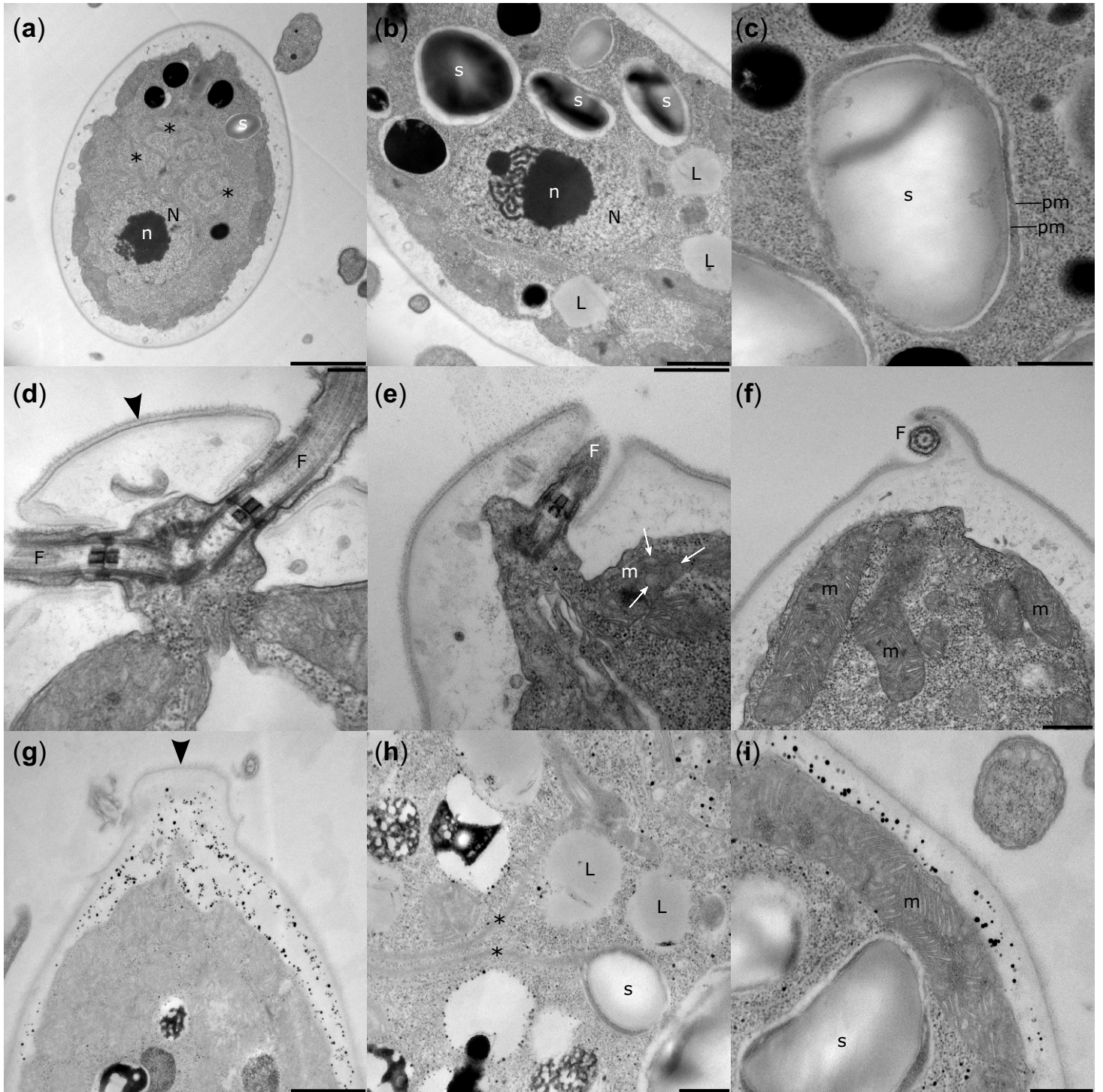


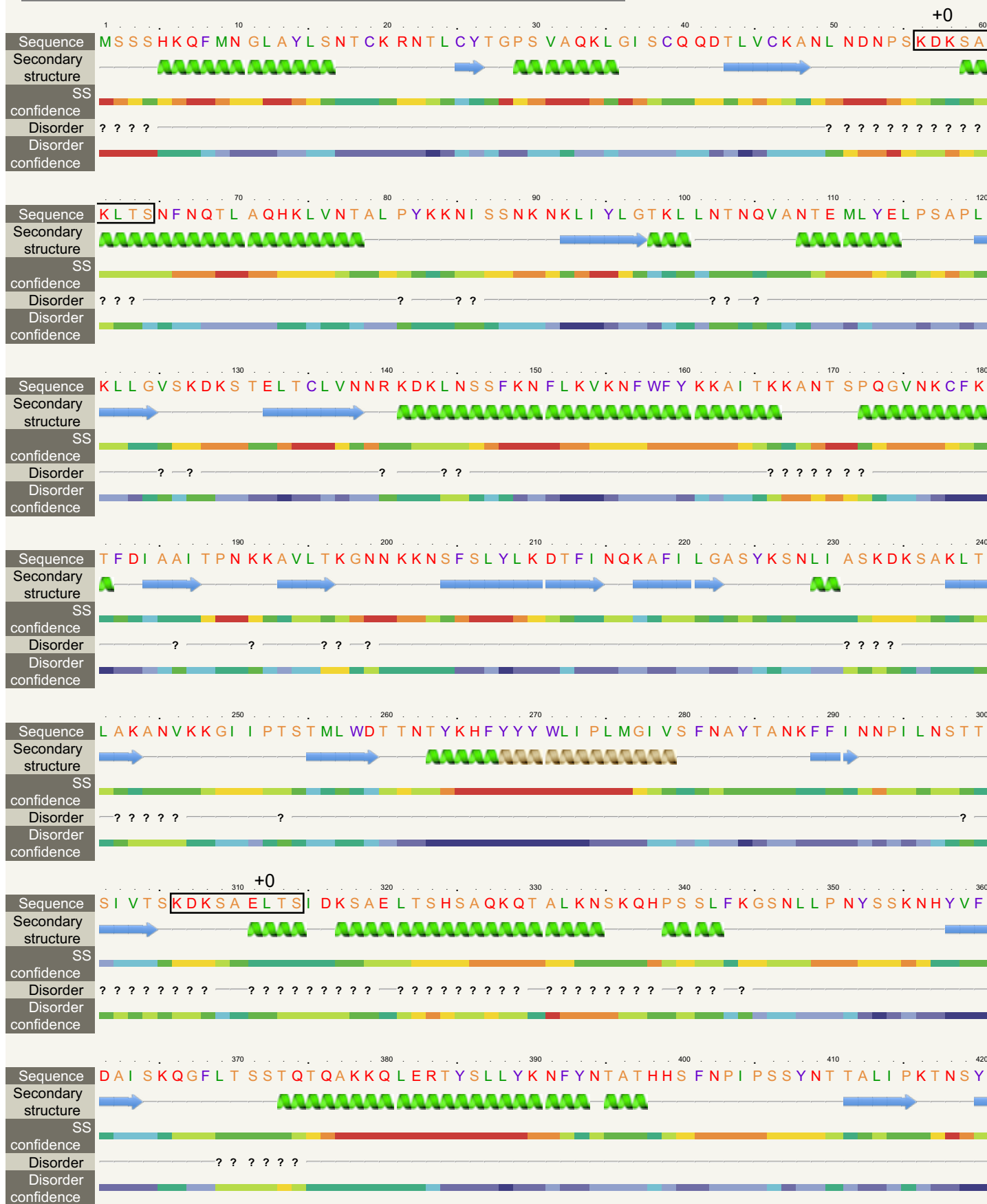
Fig. S5 Ultrastructure of *Leontynka elongata* (a–f) and *Leontynka pallida* (g–i). (a) Cell with a slightly posterior nucleus and highly convoluted leucoplast. (b) Cell with a central nucleus and multiple starch blocks. (c) Two membranes surround the plastid. (d) Prominent keel-shaped papilla and two flagella. (e) Cross section through mitochondria with discoidal cristae (white arrowheads). (f) Longitudinal section through mitochondria with discoidal cristae. (g) Ovoid cell with a keel-shaped papilla. (h) Presence of lipid droplets in an older cell. (i) Mitochondria containing putative tubulo-vesicular cristae (longitudinal section through the organelle). Abbreviations: L – lipid droplet; N – nucleus; n – nucleolus; s – starch; m – mitochondrion; F – flagellum, v – vacuole. Black arrowheads indicate papillae; asterisks mark “bridges” jointing plastid’s compartments. Scale bars: a = 2 μm ; b, g = 1 μm ; c, f, h, i = 0.5 μm ; d, e = 0.2 μm .

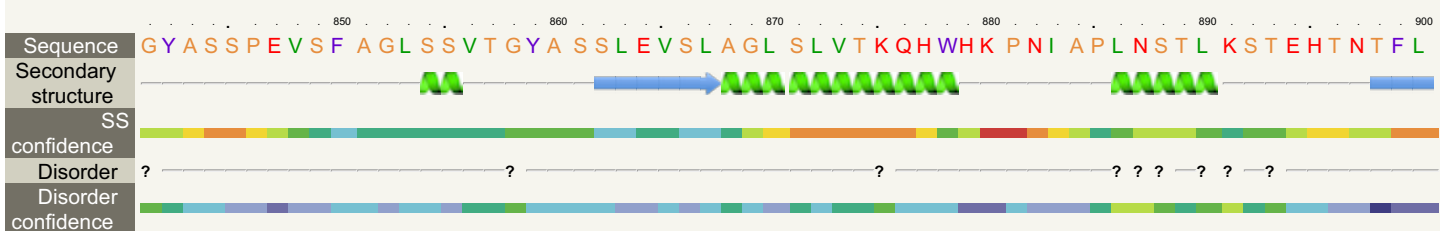
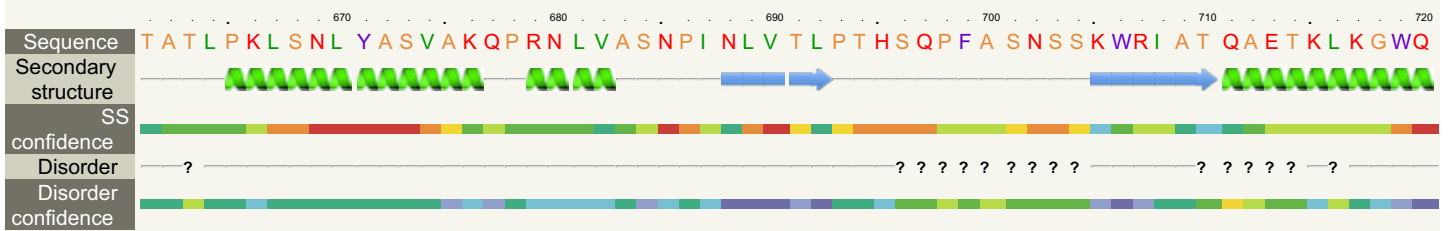
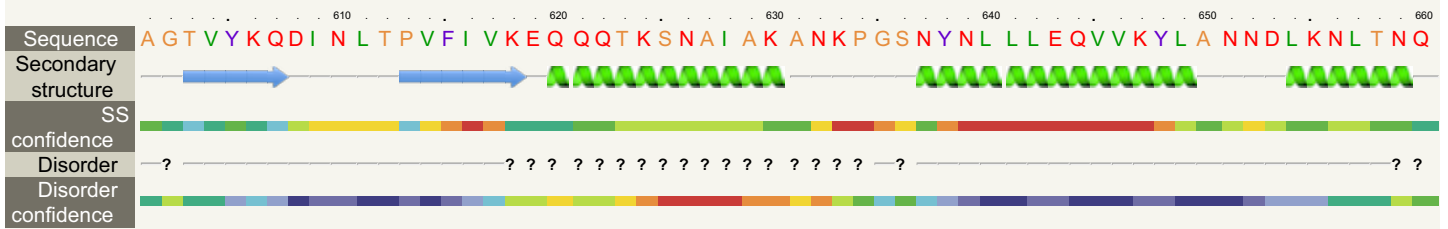
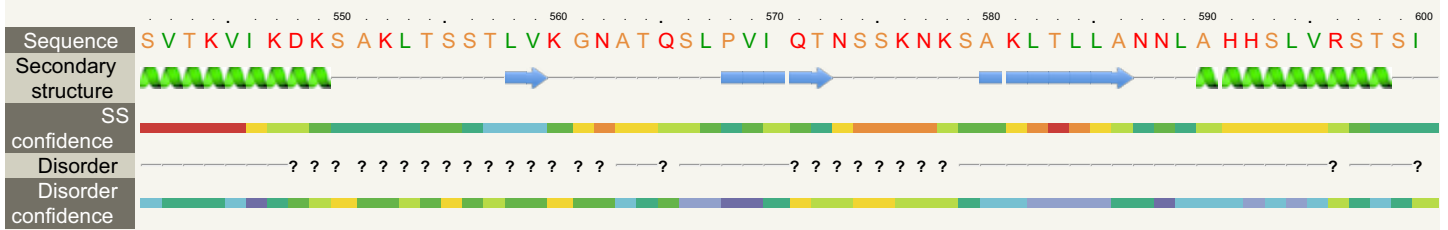
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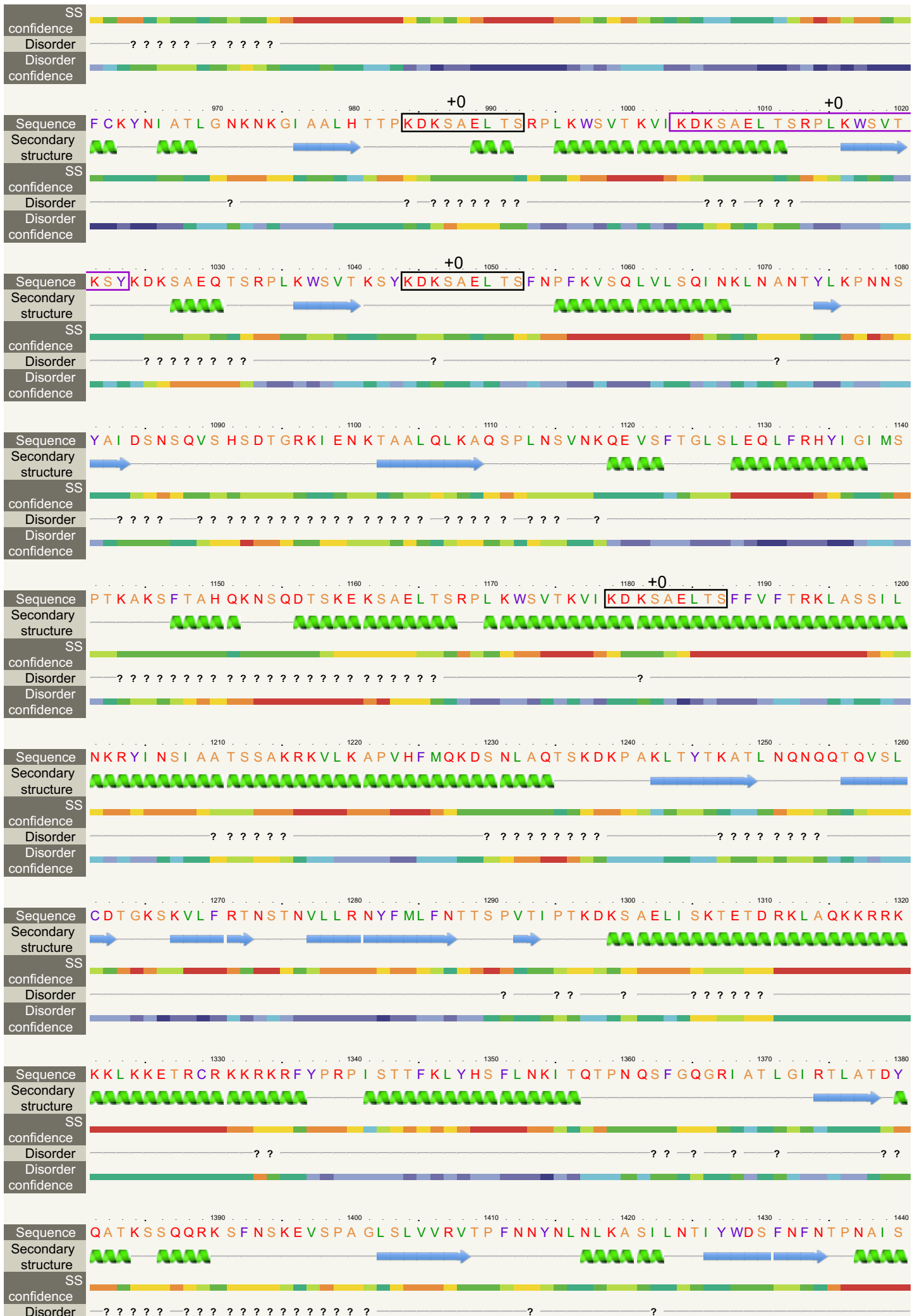
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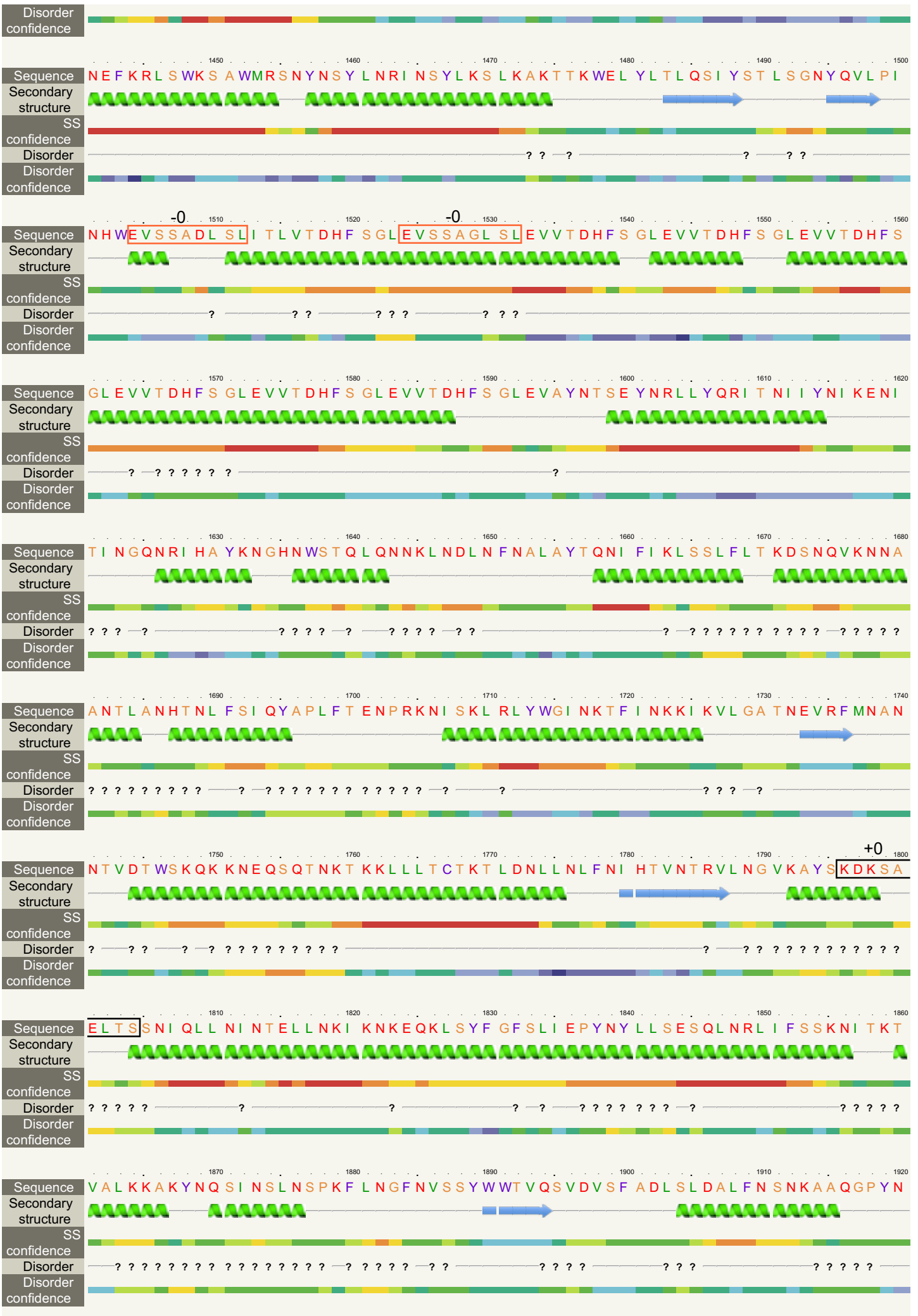
Confidence Key
 High(9) ██████████ Low (0)
 ? Disordered (30%)
 α Alpha helix (54%)
 β Beta strand (11%)
 T M helix (2%)

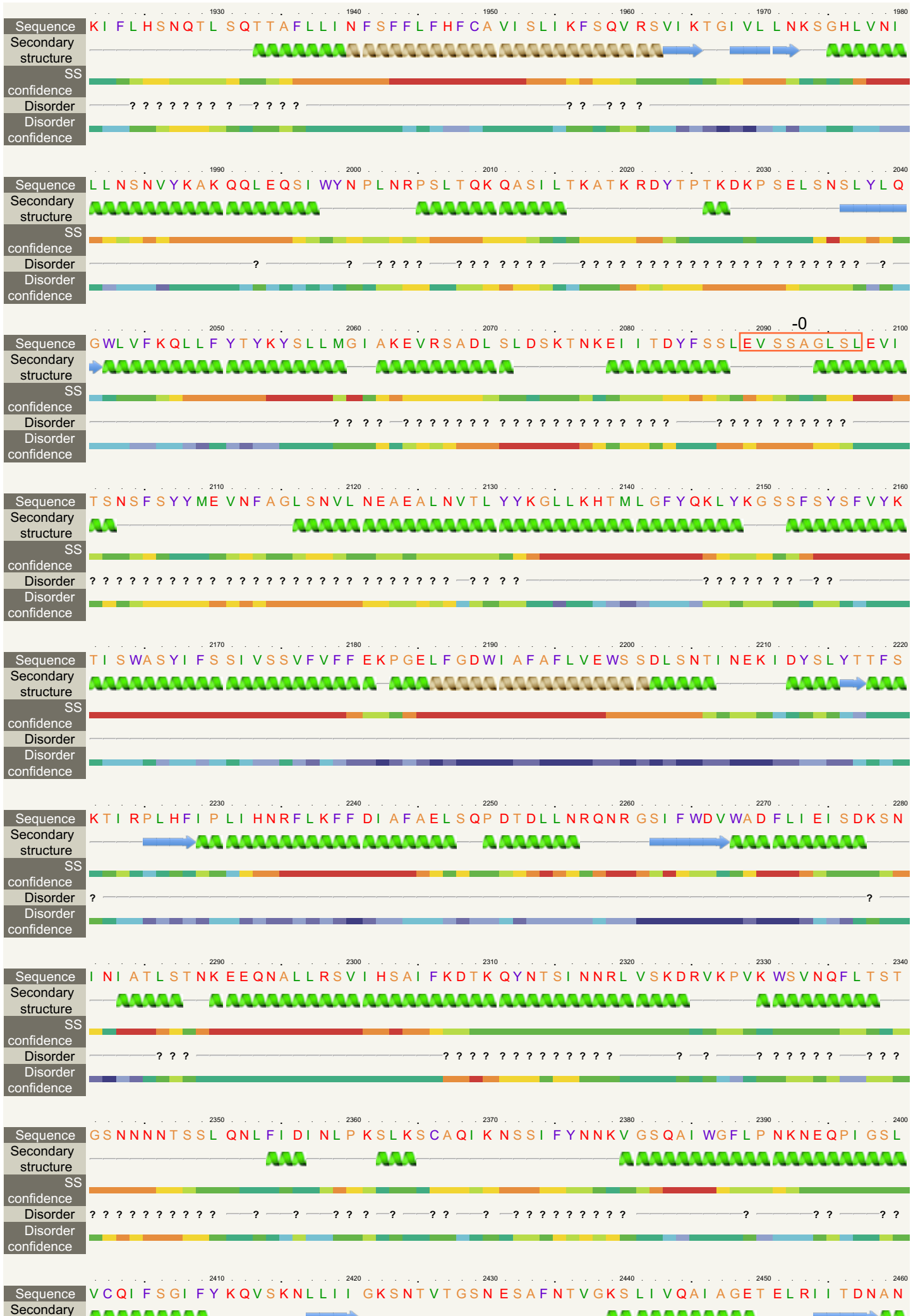
Secondary structure and disorder prediction
 FtsH - *Leontynka pallida*

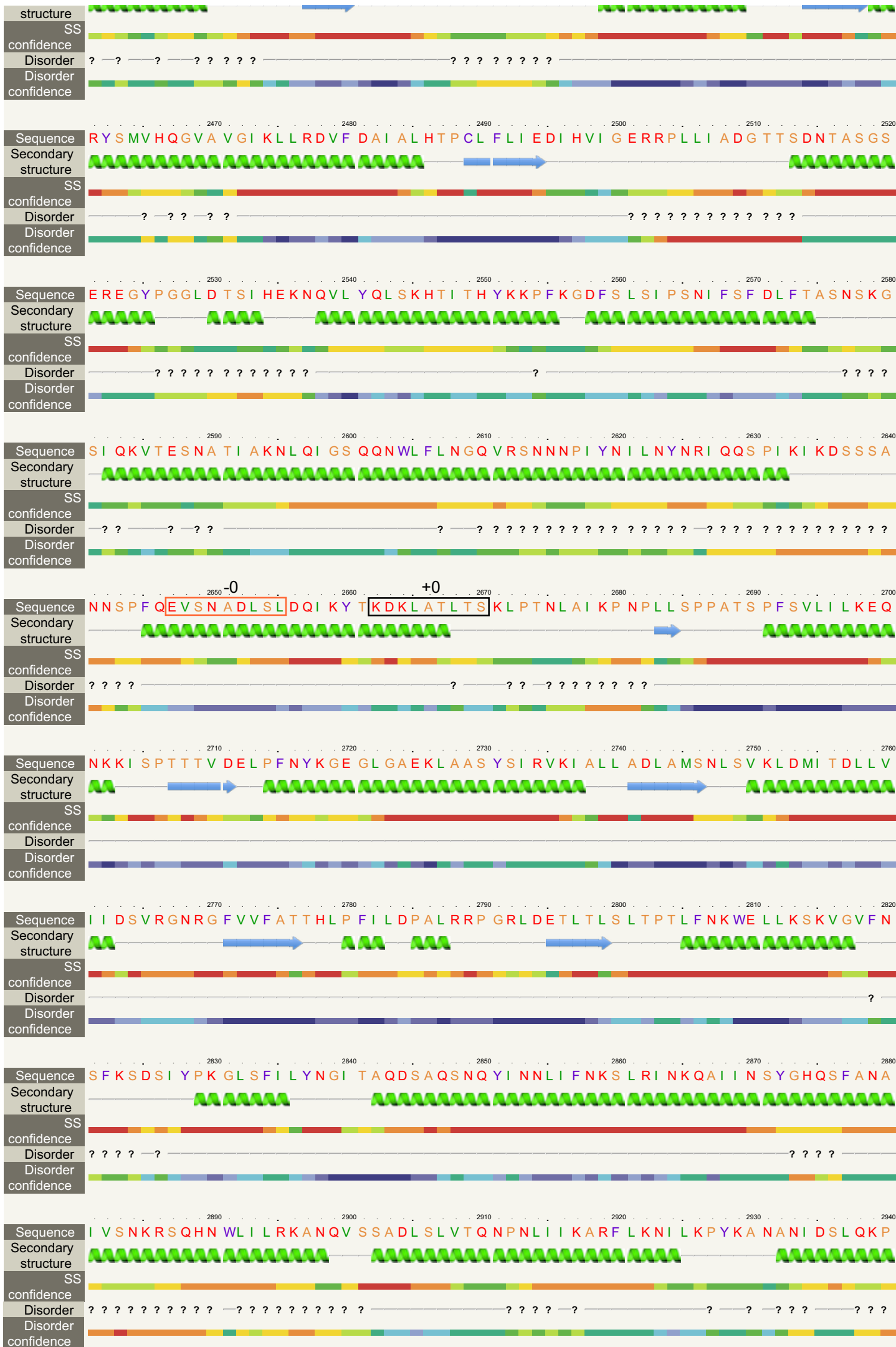












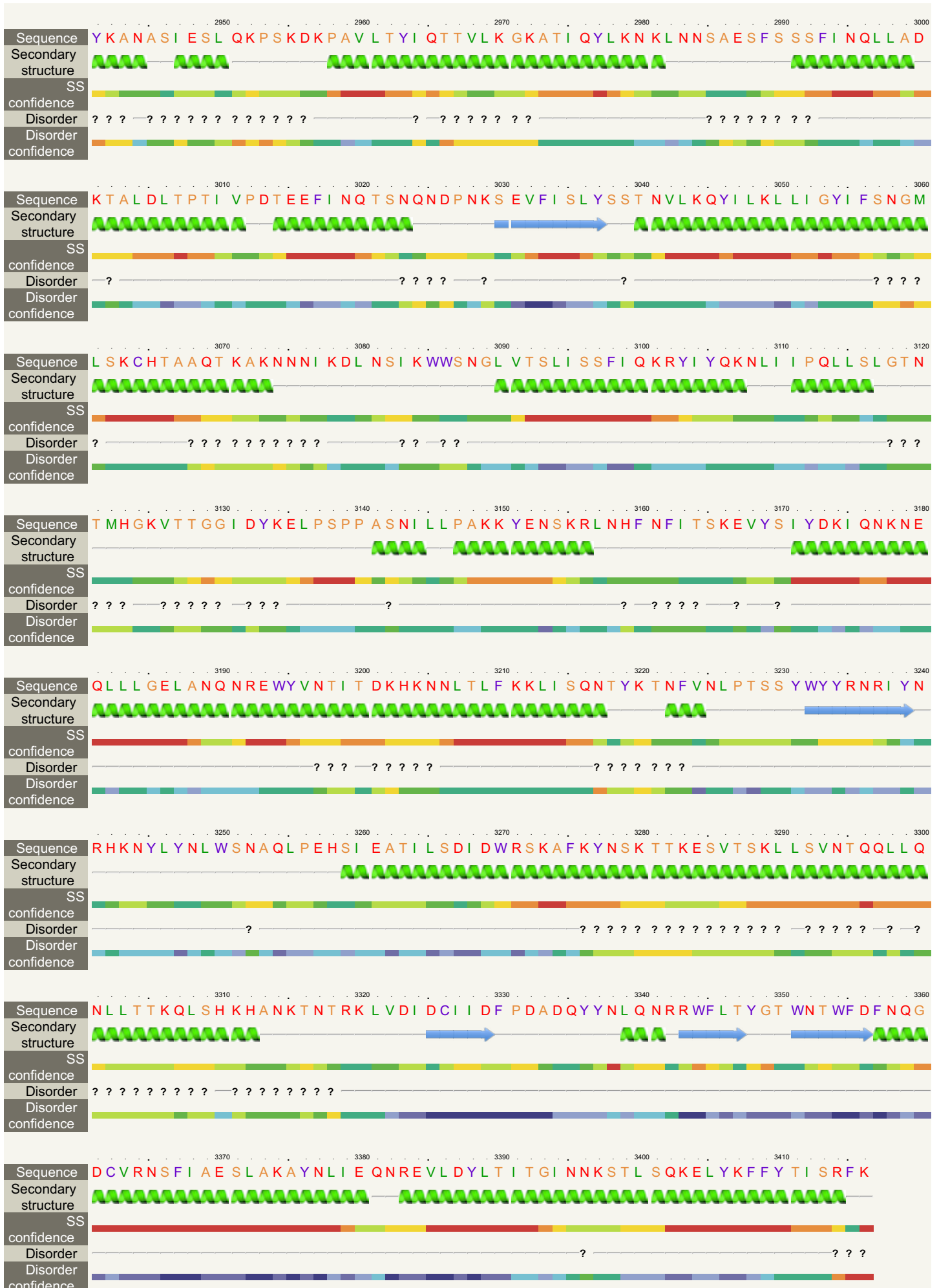


Fig. S6 Occurrence of the “variant 8” repeat (see Fig. 4) in the FtsH protein of *Leontynka pallida* mapped on its predicted structure. Protein model has been computed *in silico* using Phyre2 program. The „variant 8” repeat in RF +0 and -0 as well as a larger repeat containing the „variant 8” repeat in RF +0 are highlighted in coloured boxes.

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realigned-end
mtDNA_final CTCTCAACATGTTCTTAAACATGTTCTCCTAACATGTTCCAGAGAACATGTTCTCTTGGCTCGTCCGAGTCGCTCGGCTTCGCCCTCGCTCCTCG

210 220 230 240 250 260 270 280 290 300
realigned-end
mtDNA_final TCCGAGCCCCATGTTCTTGCACTTTGTGAGATGCTTTCCCAAGCCCCAACATGTTCTCTTAAACATGTTCTCTTAAACATGTTCTCTTGGCTCGTC

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realigned-end
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realigned-end
mtDNA_final TGGCTCGTCCGAGTCGCTCGGCTTCGCCCTCGCTCCTCGTACGAGCCAGGTCATGTCCTAACCCGTTCCCGGAACCGTTCCCGGAACCGTTCCCGGA

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realigned-end
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realigned-end
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realigned-end
mtDNA_final CGTTCGCCGGAACCGTTCCCGGAACCGTTCCCGGAACCGTTCCCGGAACCGTTCCCGGAACCGTTCCCGGAACCGTTCCCGGAACCGTTCCCGGA

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2010 2020 2030 2040 2050 2060 2070 2080 2090 2100
realigned-end
mtDNA_final

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realigned-end .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
mtDNA_final   ACGGTAAGAGGGCAACGGTAAGAGGGCAAACGGTAAGAGGGCAACGGTAAGAGGGCAACGGTAAGAGGGCAACGGTAAGAGGGCAACGGTAAGAGGGCAAC
      5710      5720      5730      5740      5750      5760      5770      5780      5790      5800
realigned-end .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
mtDNA_final   CAGAGACAACATGTTCTCTTGTGGCTCGTCCGAGTCGCTCGGCTTCGCTTCGCTTCGCTTCGCTTCGCTTCGCTTCGCTTCGCTTCGCTTCGCTTC

```

Fig. S7 Alignment of the highly similar terminal regions of the originally assembled linear mitogenome contig. The sequence similarity of the termini is 97.7% (along the alignment containing 5,771 nucleotide positions). The sequence marked here as “mtDNA_final” represents the variant of the terminus that was retained in the final putatively circular-mapping full mitogenome sequence.

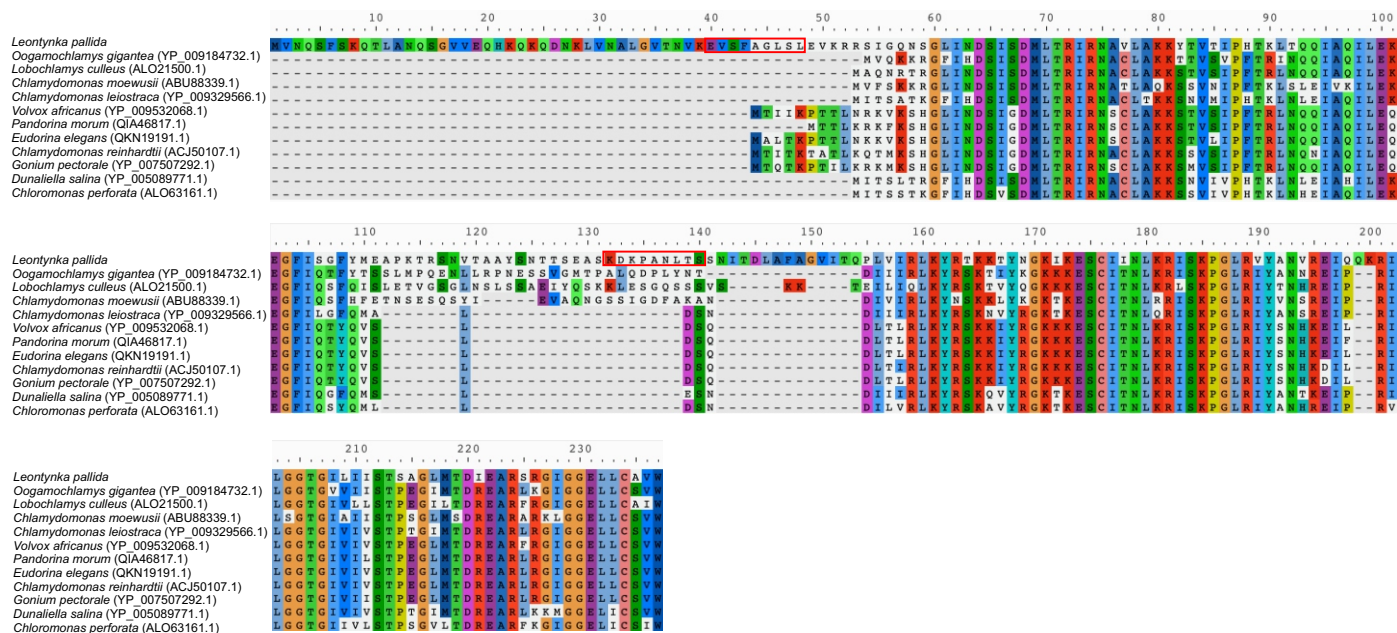


Fig. S8 Occurrence of the “variant 8” repeat (translated in reading frame +0 as KDKPANLTS and -0 as KEVSFAGLSL; both boxed in red) in variable region of protein sequence of the ribosomal protein Rps8 from *Leontynka pallida* (full protein alignment together with representatives of other chlamydomonadalean algae).